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Preliminary Report on Olfactory Neurons Specific to the Sex Pheromone of the American Cockroach

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Preliminary Report on Olfactory Neurons Specific to the Sex Pheromone of the American Cockroach. Minoru YAMADA* (Fisheries Laboratory, Faculty of Agriculture, Nagoya University, Nagoya) Shoziro ISHII, Yasumasa KUWAHARA (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto) Received February 15, 1968, *Botyu-Kagaku*, 33, 37, 1968.

5. ワモンゴキブリ性誘引物質に特異的な嗅覚中枢神経に関する予報. 山田 稔 (名古屋大学農学部水産学教室) 石井象二郎・桑原保正 (京都大学農学部附属農薬研究施設) 43. 2. 15 受理

ワモンゴキブリの性誘引物質は雄のみを激しく興奮させ、誘引する。しかし電気生理学的な実験では雄・雌・幼虫いずれの触角からも EAG (electroantennogram) を惹起する (Boeck 等 1963)。中枢神経系における電気生理学的な実験の結果、性誘引物質に対して特異的に反応する神経が雄・雌共中大脳 (deutocerebrum) に存在するらしい。

Introduction

Although the chemical structure of the sex pheromone of the female American cockroach, *Periplaneta americana* (L.) still remains in question^{1,2,3}, the male adult of this species can detect the sex pheromone with its olfactory sense organ in the antennae⁴. However, the recording of the summated receptor potentials (electroantennograms) from the American cockroach showed that the sex attractant elicited electrical responses not only in the antennae of the male but also in those of the female and nymph⁵. The present experiments have been undertaken in order to study preliminary whether there are neurons specific to the sex pheromone in the olfactory center of the cockroach.

Materials and Methods

The adults of the American cockroach bred in the laboratory were used. An unanaesthetized cockroach was fixed with adhesive tapes and wire hooks on a cork plate so that the head could not be moved. The brain was approached dorsally through the frons, and the minimum amount of the overlying tracheation was removed. Recording of the electrical responses was made extracellularly by means of a glass capillary electrode filled with 3M KCl solution. The electrical resistance of the electrode ranged between 30M Ω and 50M Ω .

The electrical activity of the neurons in the deutocerebrum was fed into a cathode follower and then into a conventional d-c and a-c amplifier. Under these conditions it was possible to record the nerve activity for many hours or even one day. Stimulation was accomplished by puffing odorous substances contained in polyethylene squeeze-bottles.

The prepurified sex pheromone used in the present experiment was extracted by the following procedure modified from Wharton *et al*⁶. Some one hundred virgin females were fed on dog biscuits and water in a glass pot in which filter papers were placed. After two weeks the filter papers were soaked into 500ml of water. The water extract was steam-distilled until the distillate reached to 1000ml. After salting out the sex pheromone was extracted with 500ml of ether. The ether layer separated was kept at -20°C until use. This ether solution of the prepurified sex pheromone showed strong activity to elicit sexual response of the male cockroach when a filter paper impregnated with this solution was brought close to the cockroach, whereas no response occurred to the female and nymph. The concentrated crude sex pheromone dissolved in ether was dropped onto a filter paper and used as a stimulant after evaporation of the solvent. Odor stimuli were applied at regular intervals during the course of electrode penetration into the brain in order to ensure that the electrode did not miss the units that were not spontaneously active.

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Results and Discussions

Figure 1 shows the examples of the single unit activity of the male cockroach. Propionic acid induced a positive slow potential (upward deflection) which was sustained during the stimulation, while the discharge of impulses was depressed. After the cessation of stimulation the units exhibited an increased activity (Fig. 1 A). It is interesting to note, on the other hand, that the prepurified sex pheromone (Fig. 1 B) and methylethyl ketone (Fig. 1 C) produced a negative slow potential (downward deflection) accompanied by a burst of activity which was

followed by a silent period and a gradual increase to the normal background activity. These changes in activity may be explained in terms of hyperpolarization or depolarization of the membrane. These units that are sensitive to both the sex pheromone and other odors were frequently found in both male and female of this insect.

Figure 2 shows the responses of the highly specific neurons to odor stimuli. The recordings were made from the same male cockroach as that used in Figure 1. The unit was not spontaneously active, but responded vigorously to the sex pheromone with a slow negativity accompanied by a train of impulses during the

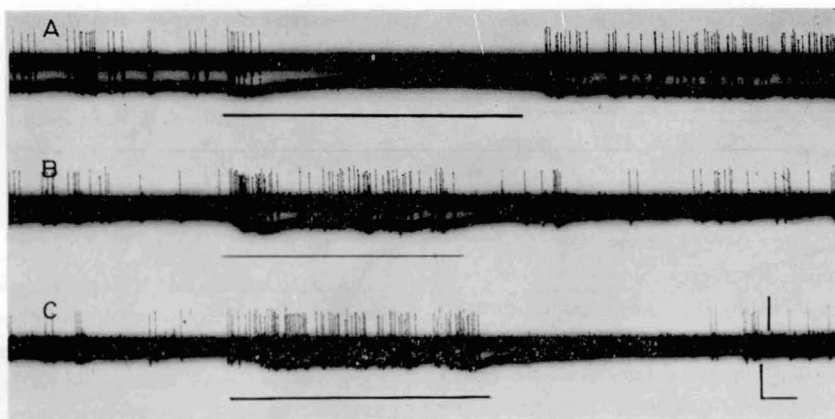


Fig. 1. Responses of a neuron in the deutocerebrum of the male cockroach to propionic acid (A), prepurified sex pheromone (B), and methylethyl ketone (C). Upper tracings are AC recordings (calibration 2mV), and lower tracings DC recordings. Time calibration 200msec. The downward deflection represents negative potential at the recording electrode. The black bars below each tracing indicate the duration of the stimulation.

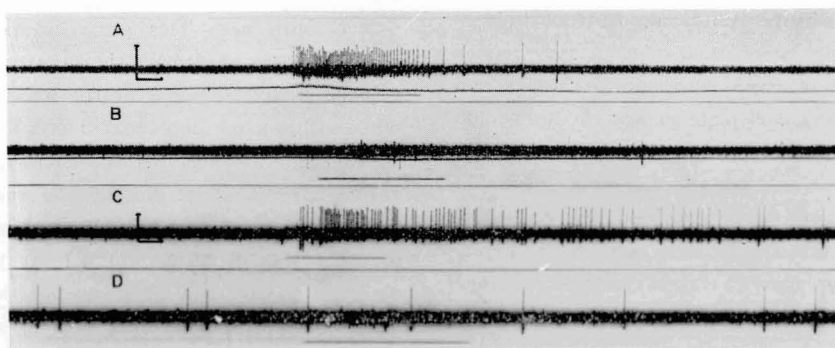


Fig. 2. Responses of a neuron in the deutocerebrum of the male cockroach to the prepurified sex pheromone (A) and methylethyl ketone (B), and those of the female cockroach to the prepurified sex pheromone (C) and methylethyl ketone (D). Calibration: 1 mV, 200 msec. in A and B; 0.5 mV, 200 msec. in C and D.

stimulation (Fig. 2 A). However, it failed to respond to other types of odor stimuli tested, for examples, propionic acid, acetic acid, benzyl acetate, *p*-dichlorobenzene, 2-methyl-2-butanol, acetophenone, geraniol, 1,2-dichloroethane, trichloroethylene, cycloheptanone, diethylsulphate, cyclopentanone, ethyl ether, methyl alcohol, methylethyl ketone. A neuron of the female also showed such a high specificity to the prepurified sex pheromone as in the male (Fig. 2 C and D). However, this unit showed spontaneous discharges.

Because of the stimulations with the prepurified sex pheromone and the small number of odorous chemicals tested, it is difficult to draw the conclusion about the "Sex pheromone neuron". Although the present experiments were failed to elucidate that the sex pheromone elicits sexual response only to the male and not to the female and nymph by electrophysiological method in the neuron of deutocerebrum of both sexes, it is of interest that the present finding together with the responses at the receptor level⁹⁾, suggests a possibility that these extremely specific units in both sexes may play an important role in response to the sex pheromone.

If so, it may be considered that there it no discrimination in response to the sex pheromone in the neuron of deutocerebrum between male and female of the american cockroach.

Acknowledgement

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Studies on the Increment of the Efficacy of Insecticides (VII). On the Effect of Synergized Pyrethroid on Various Strains of House Flies. Akifumi HAYASHI and Masayoshi HATSUKADE (Laboratory of Applied Entomology, Taisho Pharmaceutical Co., Ltd. Toshima-ku, Tokyo) Received February 8, 1968. *Botyu-Kagaku*, 33, 39, 1968 (with English Summary. 5)

6. 殺虫剤の効力増進に関する基礎的研究 (VII). 各種の系統イエバエにおける共力剤を加えたピレスロイドの効果について. 林 晃史, 廿日出正美 (大正製薬株式会社防虫科学研究室) 43, 2, 8 受理

7系統のイエバエ *Musca domestica* に対し, アレスリン, ピレトリンおよびフタルスリンを単用あるいは S-421 およびピペロニルブトキサイドを加用して局所施用し, 殺虫力を比較した. 一般に *M. d. domestica* は *M. d. vicina* に比べてピレスロイドに対する抵抗力が強かった. また供試ピレスロイドに共力剤を加用することにより7系統のイエバエに対する殺虫効力が増強された.

先に, 林, 廿日出(1967)³⁾は系統のことなるイエバエにおけるピレスロイドに対する感受性の相違について報告した. 本実験ではさらに3系統を追加しピレスロイドに抵抗性を示す系統に対する共力剤の影響についてしらべ, ヨーロッパ系は本邦系にくらべてピレスロイドに対して強いことを確認するとともに, 共力剤を加用することによって抵抗性を打破し得ることを知ったので報告する.

本文に入るに際し, 御指導いただいた名古屋大学農学部弥富三教授および斎藤哲夫助教授, また, しば

しば有益なる御助言を賜った東京医科歯科大学加納六郎教授および国立予防衛生研究所安富和男博士に御礼申しあげる. なお, 発表を御快諾いただいた上原昭二副社長, 常務取締役井川俊一博士, および研究部長田中一郎博士に謝意を表する.

I. 実験材料および方法

(1) 供試薬剤 この実験に用いた有効成分はピレトリン (25% エキス), アレスリンおよびフタルスリンの3種でいずれも工業用である. また, 共力剤は